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An In silico Approach for Molecular Targets in *Candida albicans* using Prodigiosin-a Bacterial Pigment for Anti-fungal Activity

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ABSTRACT: Drug resistance in *Candida* sps especially *Candida albicans* led to increased morbidity and mortality in mankind all over the world. The development of different antifungal drugs with novel targets is the need of the day. The unicellular *Candida* species are opportunistic pathogens causing simple mucocutaneous to fungemia leading to death in immunocompromised patients. Among different infections caused by *Candida,* cutaneous candidiasis which is an infection of the skin is alarming. This fungus has survival and virulence factors leading to decreased host immunity response making infection more difficult to control. The increase in fungal resistance necessitates the search for novel antifungal drugs with different mechanisms of action. Hence, in the present investigation, an attempt was made *in silico* using prodigiosin a bacterial pigment as a ligand to identify different molecular targets in *Candida albicans* for antifungal activity using fluconazole as a standard reference drug. An advanced docking software Auto Dock was used for the study. Prodigiosin was found to show potent binding affinity to Sterol 14-alpha demethylase (CYP51) followed by Secreted aspartic proteinase (Sap) 5 and Als3 adhesin from *Candida albicans* compared to the standard reference drug fluconazole.

From the above results, it can be concluded that prodigiosin can be a potent drug in treating dermatological problems caused by *Candida* sps. However, *in vitro* and *in vivo* studies are needed for confirmation of Prodigiosin activity.

Keywords: Fluconazole, Prodigiosin, Sterol 14-alpha demethylase (CYP51), Secreted aspartic proteinase (Sap) 5, Als3 adhesin.

INTRODUCTION

Human skin which harbors a variety of microorganisms *viz*., bacteria, fungi and viruses acts as a physical barrier preventing the invasion of pathogens. Under certain conditions, this barrier breaks resulting in skin or systemic infections (Byrd *et al*., 2018). These invasive fungal infections are a progressively more common threat to mankind all over the world. Among fungal infections, *Candida* species are the leading cause of a 40% mortality rate globally every year (Tan *et al*., 2021). In *Candida* species *viz*., *Candida glabrate, Candida tropicalis, Candida parapsiosis and Candida kruse*, *Candia albicans* alone are responsible for 50% of *Candida* infections (Tan *et al*., 2021). The fungus *Candida* is a normal flora of not only the skin but also the vagina, oral, and gastrointestinal tract (Calderone, 2002) causing superficial skin and mucosal infections in healthy individuals and invasive fungal infections in Immunocompromised patients resulting in deep penetration and systemic infections like blood, urinary and nervous system candidiasis with fatal outcome (Vazquez-Munoz *et al*. 2021). The fungus *Candida albicans* is present in yeast form in the human microbiome and undergoes a transition to hyphal form which is pathogenic (Talapko *et al*., 2021). This ability of transition is a crucial factor for the virulence of *C. albicans* which includes the secretion of enzymes, adhesion to the cell surface and evasion of the immune system (do Nascimento Dias *et al*., 2020). For survival under harsh environmental conditions like exposure to antifungal drugs and disinfectants, *Candida* species also form biofilms on tissues and abiotic surfaces (Monoz *et al*., 2020) which protects the fungus against immune cells and increase resistance to antimicrobial drugs and other physical, chemical and environmental stress (Sari *et al*.,2019; Lohse *et al*., 2018). Polymorphism and the ability to form biofilm are the two major virulence factors of *Candida albicans* (do Nascimento Dias *et al*., 2020). The development of single and multidrug resistant *Candida albicans* strains has been identified recently (Bitew and Abebaw 2018; Canela *et al*., 2018; Khedri *et al*., 2018). The fungus *C. albicans* have developed resistance to most commonly prescribed antifungal drugs like fluconazole, other azoles, minocycline, echinocandins etc. (Lee *et al*., 2021) which necessitates the search for new and highly effective antifungal drugs and new targets which are safe and economical.

Microbial pigments which are microbial metabolites would be the best alternative to address this issue. These pigments are colored molecules with the absorption of light at specific wavelengths, and diverse chemical components with potential biological activities (Kim, 2013) *viz*., cytotoxic, antioxidant, antimicrobial, antimicrobial, anticancer and other activities (Ramesh *et al*., 2019).

Hence, in the present investigation, different proteins of *Candida albicans* were targeted for anti-*Candida a*ctivity using Prodigiosin, a bacterial pigment *in silico*.

MATERIALS AND METHODS

Preparation of Ligands. In the present study, Prodigiosin-a bacterial pigment and fluconazole an antifungal drug were used as ligands. The structures of the ligands were drawn using Chem Draw and were converted to 3D PDB format from mol format using an online conversion tool https://cactus.nci.nih/translate/. The hydrogen atoms were added and energy minimization of the ligands was done and saved to pdbqt format using Autodock software 4.2 version software tools.

Preparation of Proteins. The three dimensional structures of the target proteins *viz.,* Sterol 14-alpha demethylase (CYP51), Secreted aspartic proteinase (Sap) 5 and Als3 adhesin from *Candida albicans* were downloaded from the Protein database (https:\\www.rcsb.org/) with PDB ID's 5TZ1, 2QZX and 4LEB respectively in PDB format. Later, the water molecules and bound ligands were removed from the proteins and missing atoms were corrected, Kollmann charges and hydrogen atoms were added, energy minimization was done and later converted to pdbqt format using auto dock software.

Software Validation. The Auto dock was validated before performing the docking of prodigiosin by downloading the X-ray crystal structure of the receptors viz. Sterol 14-alpha demethylase (CYP51) (PDB ID:5TZ1, Secreted aspartic proteinase (Sap) 5 (PDB ID:2QZX) and Als3 adhesin (PDB ID:4LEB) of *C. albicans* from the protein data bank and redocking the co-crystallized ligand reproducing the original interactions of the reference protein-ligand complexes comparing the root-mean square distance of the experimentally determined pose with the docked pose.

Molecular Docking. After preparing the selected ligands and receptors they were converted to pdbqt format using auto dock software. To identify the best target site for prodigiosin, the molecular docking was performed with Sterol 14-alpha demethylase (CYP51) (PDB ID: 5TZ1), Secreted aspartic proteinase (Sap) 5 (PDB ID: 2QZX) and Als3 adhesin protein (PDB ID: 4LEB) of *C. albicans* using autodck 4.2 **(**https://autodock.scripps.edu/**).** A grid box was prepared for each protein to cover the pocket with the main residues of the protein binding site by maintaining the grid size of X=40, Y=40 and Z=40. The coordinates used for docking the ligands with Sterol 14-alpha demethylase (CYP51) (PDB ID: 5TZ1) were x= 70.47; y=69.10; z=4.43. The coordinates used for Secreted aspartic proteinase (Sap) 5 (PDB ID: 2QZX) were x=9.73; y=32.99; z=24.50 and the coordinates for Als3 adhesin protein (PDB ID: 4LEB) were x=28.45; y=2.11; z=-18.29. An advanced molecular docking program auto dock version 4.2 was used to study the binding affinities (kcal mol^{-1}). The ligands were evaluated *in silico* against the receptors of *C. albicans* in triplicates. Based on the complete ten runs, the average of the best conformations was chosen with the lowest docking energy. The interaction of proteins with ligands, hydrogen bonds, bond length and root mean square difference (RMSD) was analyzed using the Discovery studio visualizer.

Evaluation of Drug Likeliness. Lipinski's rule of five is very useful in studying pharmacokinetic parameters like absorption, distribution, metabolism, elimination and toxicity of drugs (ADMET). This rule of five is very helpful in novel drug design and development. The drug likely ness and molecular properties of the ligand prodigiosin were done by the Swiss adme server (http://www.swissadme.ch/index.php#top /).

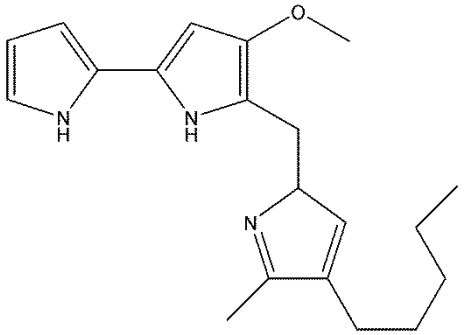
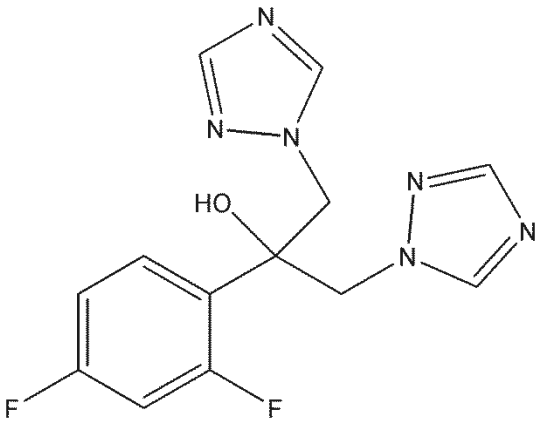
RESULTS AND DISCUSSION

Owing to the potential of microbial pigments, a bacterial pigment prodigiosin was studied for antifungal activity against different targets of *Candida albicans viz.,* Sterol 14-alpha demethylase (CYP51), Secreted aspartic proteinase (Sap) 5 and Als3 adhesin proteins using fluconazole as a standard reference drug. The computational approaches is very useful for understanding the organic compounds and their interactions with the drug targets. Molecular docking, an ecofriendly and economical process can be used in preliminary study in designing ligands and studying their interaction with the target proteins before proceeding to a wet lab is used in the study. The structures and IUPAC names of Prodigiosin and the standard reference drug fluconazole were shown in Table 1.

A. Molecular docking of Prodigiosin, a bacterial pigment with different targets of Candida albicans

Molecular docking of Prodigiosin into the active site of three proteins of *C. albicans viz*., Sterol 14-alpha demethylase (CYP51), Secreted aspartic proteinase (Sap) 5 and Als3 adhesion protein was found to be successful based on the formation of the complex of all the proteins with ligands when studied individually. The binding energies, hydrogen bond interactions, bond length and orientation of the docked compounds within the active site were visualized. The bacterial pigment under study showed the best RMSD value of 0.00 indicating a strong and favorable bonding between the proteins and ligands.

Table 1: Showing IUPAC Names and Structures of Prodigiosin and the standard Reference drug Fluconazole.

Compound.	IUPAC Name	Structure of the Compound
Prodigiosin	4-Methoxy-5-[(*Z*)-(5-methyl-4-pentyl-2*H*-pyrrol-2-ylidene)methyl]-1*H*,1′*H*-2,2′-bipyrrole	
Fluconazole	2-(2,4-Difluorophenyl)-1,3-*bis*(1*H*-1,2,4-triazol-1-yl)propan-2-ol	

The binding energies recorded for Sterol 14-alpha demethylase (CYP51) with prodigiosin and the standard drug fluconazole were found to be -9.0 and -7.1 respectively whereas, the binding energies for secreted aspartic proteinase (Sap) 5 was found to be -7.6 for prodigiosin and -7.0 for fluconazole. Similarly, prodigiosin showed a binding score of -7.1 and fluconazole showed a binding score of -6.6 when interacted with the adhesion protein Als3 of the fungus *C. albicans.* The interaction of prodigiosin with the fungal protein sterol 14-alpha demethylase (CYP51) (PDB ID 5TZ1) was found to be due to Van Der waals forces, pi-pi stacked bonds, pi sigma bonds and no hydrogen bonds were recorded and the interaction of the standard drug fluconazole was found to be with 3 hydrogen bonds and no other bonds were recorded as recorded for prodigiosin. Similarly, the binding of prodigiosin with the fungal adhesion protein Als3 (PDB ID 4LEB) was found to be with three hydrogen bonds, Van Der waals forces etc. Whereas, fluconazole was found to have four hydrogen bonds, stacked bonds and Van Der waals forces. Similarly, the interaction of prodigiosin with the other protein secreted aspartic proteinase (Sap) 5 from *C. albicans* (PDB ID 2QZX) was found to be with four hydrogen bonds, Van Der waals forces and stacked bonds. Whereas, fluconazole showed two hydrogen bonds, Van Der waals forces and stacked bonds. The number of hydrogen bonds formed, binding energies, and residues of the catalytic site involved in the protein-ligand interaction of three receptors with ligands are shown in Table 2 and Fig. 1-3.

Table 2: Interacting amino acids, H-bonds, distance and binding scores of sterol 14-alpha demethylase (CYP51), Als3 adhesin from *Candida albicans* and Secreted aspartic proteinase (Sap) 5 from *Candida albicans* (PDB IDs 5TZ1, 4LEB and 2QZX, respectively) using Bacterial pigments Prodigiosin and reference drug Fluconazole.

Name of the Ligand	Affinity kcal/mol	Number of hydrogen bonds	Amino acids of interacting protein
1. Sterol 14-alpha demethylase (CYP51) (PDB ID 5TZ1)			
Prodigiosin	**-9.0**	**Wander wall Interactions**	**--**
Flucanozole	**-7.1**	**3**	**Tyr-A:132 Arg-A:469 Phe-A:463**
2. Als3 adhesin protein from *Candida albicans* (PDB ID 4LEB)			
Prodigiosin	**-7.1**	**3**	**Ser A:170 Asp A:169 Ser A:170**
Flucanozole	**-6.6**	**4**	**Asp A:86 Asp A:86 Thr A:222 Tyr A:225**
3. Secreted aspartic proteinase (Sap) 5 from *Candida albicans* (PDB ID 2QZX)			
Prodigiosin	**-7.6**	**4**	**Thr A:222 Asp A:86 Tyr A:225 Asp A:86**
Flucanozole	**-7.0**	**2**	**Asp A:218 Gly A:85**

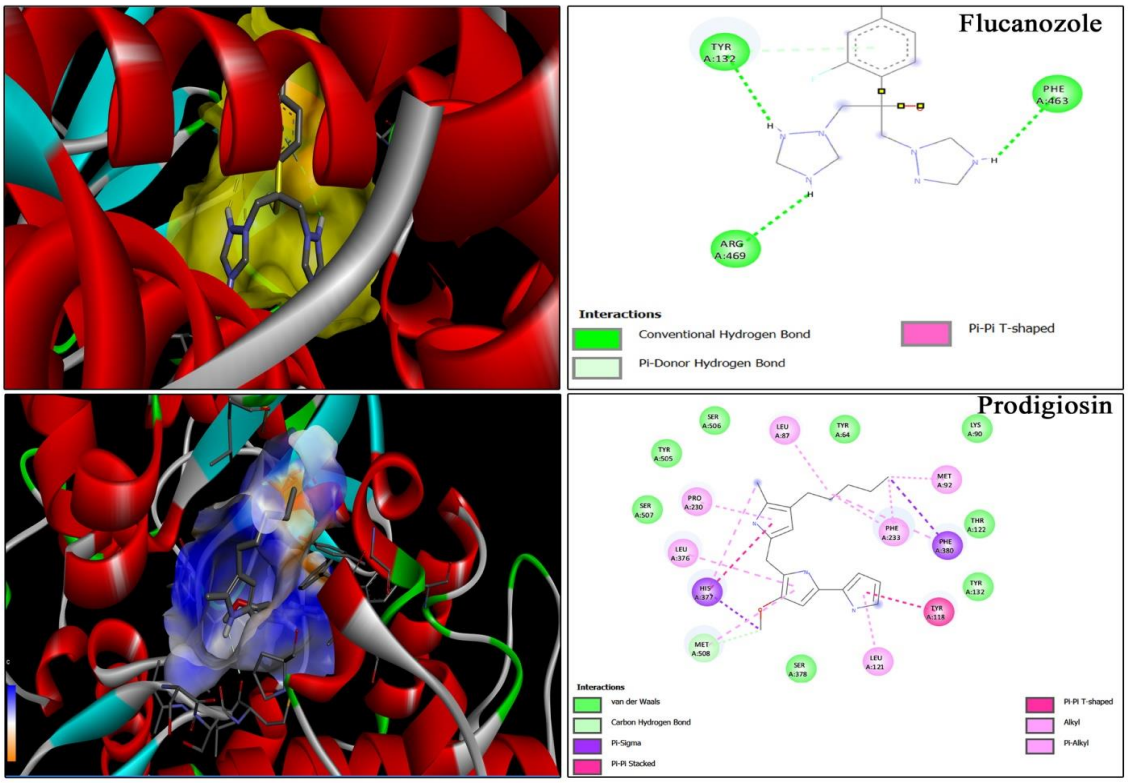


Fig. 1. Snapshot of docking of Prodigiosin with Sterol 14-alpha demethylase (CYP51) of *Candida albicans* PDB ID: 5TZ1.

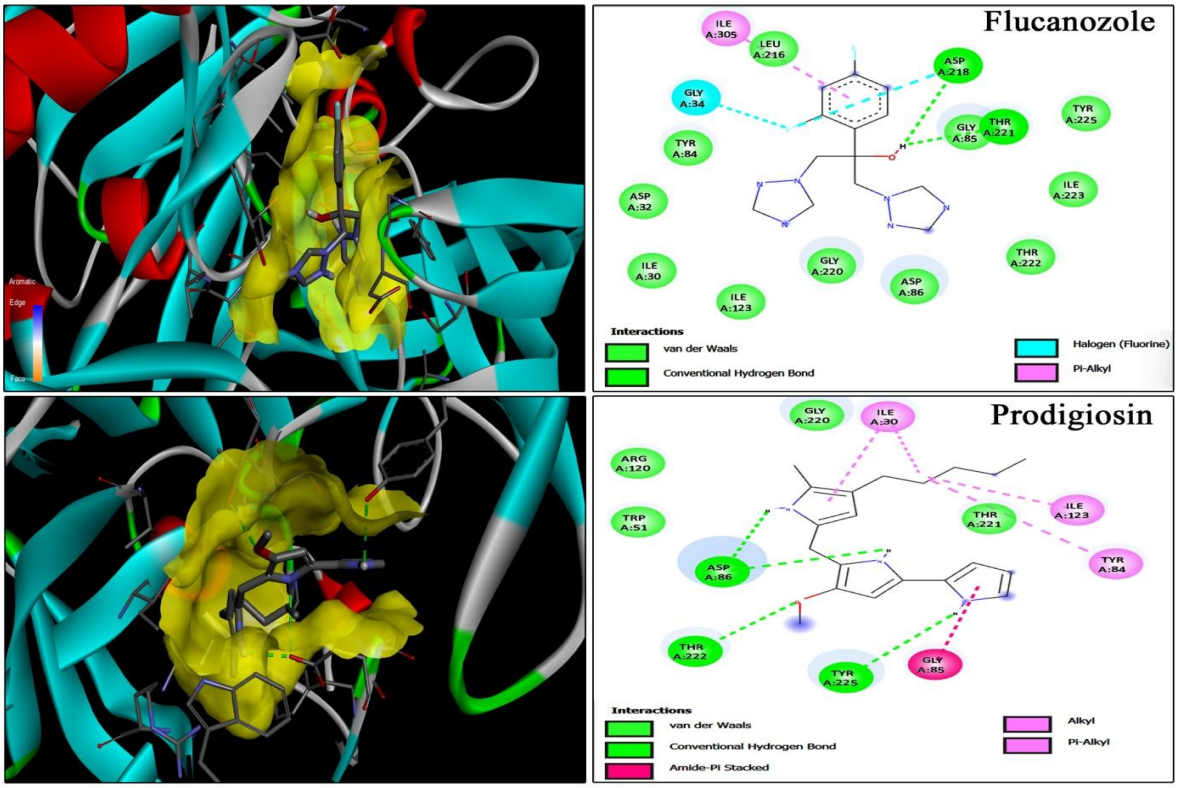


Fig. 2. Snapshot of docking of Prodigiosin with Secreted aspartic proteinase (Sap) 5 of *Candida albicans* PDB ID2QZX.

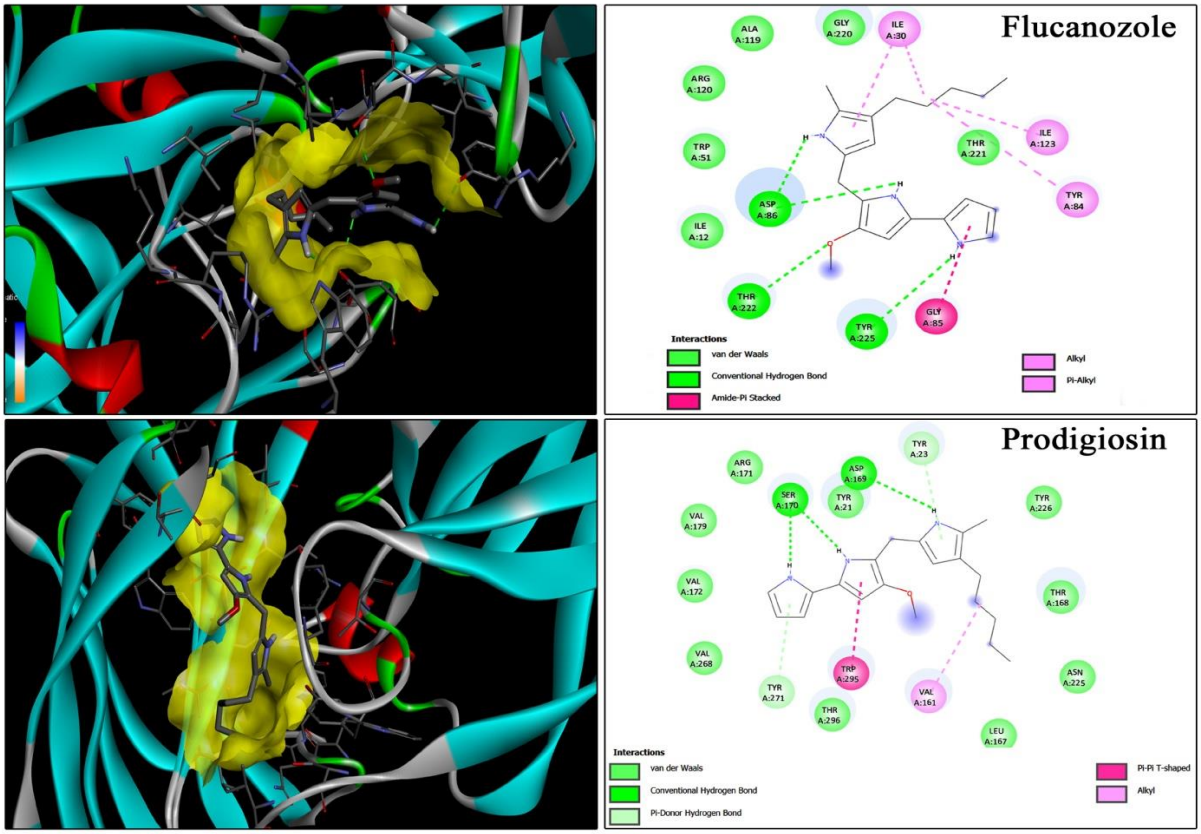


Fig. 3. Snapshot of docking of Prodigiosin with Als3 adhesin from *Candida albicans* PDB ID: 4e45.

In the present investigation, different targets of *Candida albicans viz*., Sterol 14-alpha demethylase (CYP51), Secreted aspartic proteinase (Sap) 5 and Als3 adhesin protein were selected which are responsible for the pathogenicity of *C. albicans*. In eukaryotes cells sterol 4α-demethylase (CYP51) which is a cytochrome P450

enzyme is required for the biosynthesis of sterols and is the major target of clinical drugs in treating fungal infections. Most of the antifungal drugs viz. azoles, allylamines, polyenes, morpholines and thiocarbamates use ergosterol biosynthesis as their target (Rajput and Karuppayil 2013) and are associated with high toxicity and severe side effects. In the present *in silico* study, strong inhibition of sterol 14-alpha demethylase (CYP51) by the bacterial pigment prodigiosin was observed compared to the standard drug which clearly states that prodigiosin can inhibit the proliferation of *C. albians.* Similarly, *C. albicans* upon attachment to the host will actively penetrate into the host cells and secrete specific enzyme aspartic proteinase (sap) 5 which is involved in biofilm formation (Hartanto *et al*., 2022). In the present study, prodigiosin was found to show strong binding to Sap-5 enzyme compared to standard reference drug fluconazole which clearly states that prodigiosin can prevent biofilm formation strongly compared to the standard drug. The unicellular fungus *C.albicans* also causes hematogenously disseminated and oropharyngeal candidiasis by invading host cells with the aid of the A1s3 protein (Phan *et al*., 2007). Prodigiosin showed a strong binding affinity to A1s3 protein compared to fluconazole, the standard reference drug in the study which clearly states that prodigiosin prevents host cell invasion and damage which are critical virulence factors of *C. albicans*.

B. Evaluation of drug Likeliness

Preliminary investigations related to toxicity, absorption, distribution metabolism and elimination of the drug and its metabolites are to be performed before use in humans in the process of drug discovery which can be evaluated by Lipinski's rule (RO5). The oral activity of a novel drug is predicted by calculating certain parameters like polar surface area, number of hydrogen bond acceptors, and molecular weight, number of hydrogen bond donors, log P (partition coefficient). In the present study, the bacterial pigment prodigiosin was found to be in good agreement with the given criteria and can be said to possess good oral bioavailability if orally formulated. Evaluation of drug likeliness based on Lipinski's rule of ligands was shown in Table 3 and Fig. 4.

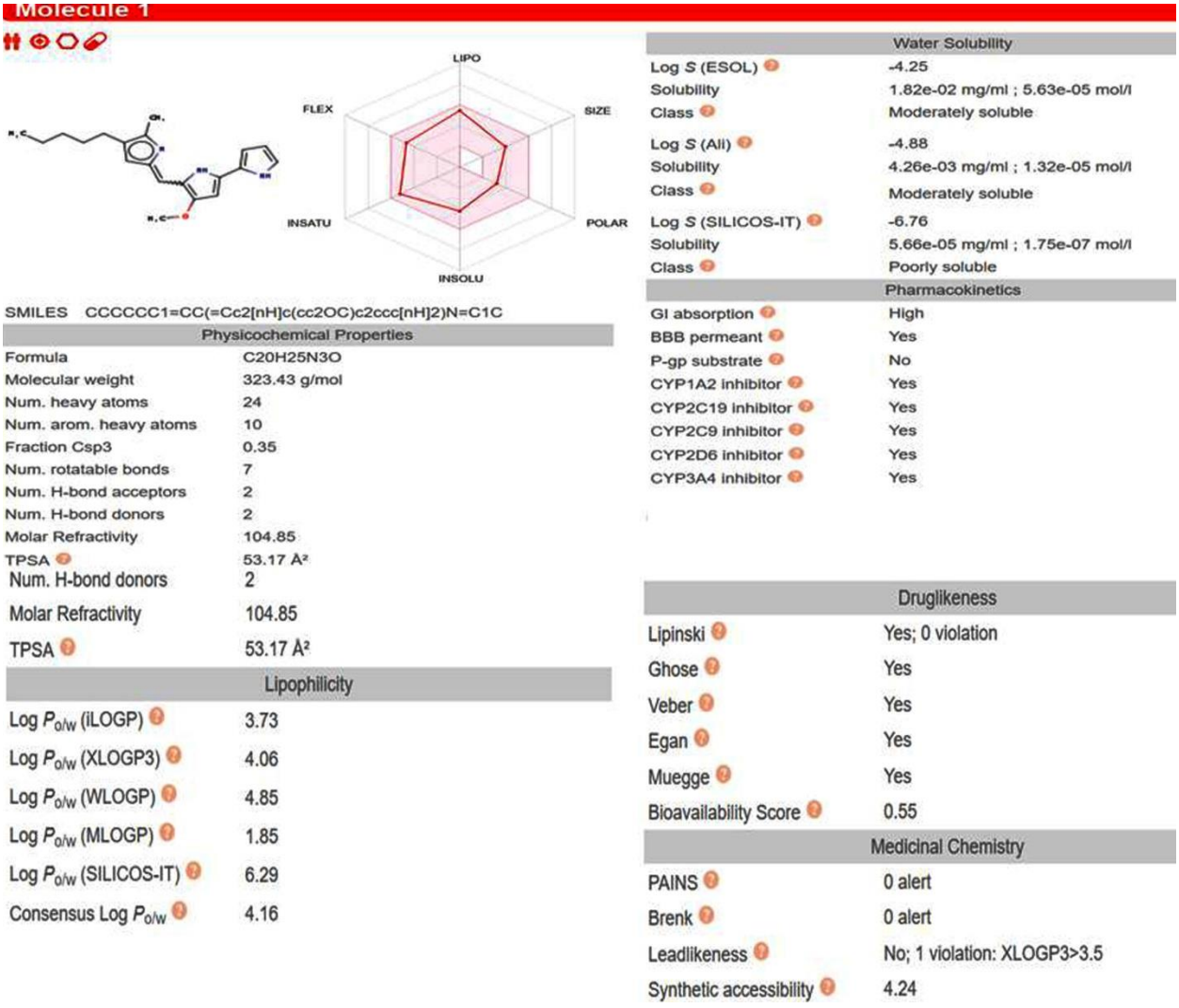


Fig. 4. Showing drug likeliness of prodigiosin.

Table 3: Showing drug likeliness of Prodigiosin in comparison to Fluconazole.

Drug likeliness Properties of nitrile compounds	MW g/mol	Consensus Log Po/w	No. of H-bond Acceptors	No. of H-bond Donors	Molar Refractivity	Lipinski	Veber	Bioavailability Score	Synthetic accessibility (SA)	TPSA (Å2)	No of rotatable bonds	Solubility class
Prodigiosin	323.43	4.16	02	02	104.85	0	Yes	0.55	4.24	53.17	07	Poorly soluble
Fluconozole	306.27	0.88	07	01	70.71	0	Yes	0.55	2.45	81.65	05	Soluble

CONCLUSIONS

Development of drug resistance by different fungi is an alarming state and there is an urgent need for novel drug discovery in an ecofriendly and economical way by identifying different fungal targets. In the present study prodigiosin, a bacterial pigment was found to superior in binding to different protein targets of *Candida albicans* compared to the standard reference drug Fluconazole and the drug likeliness was also was in compliance with the given criteria for oral formulations. Hence, Prodigiosin can be developed as an antifungal drug in treating infections of *Candida albicans*. However, further investigations are required *in vitro* and *in vivo* in confirming these results.

FUTURE SCOPE

Prodigiosin, a wonder bacterial pigment has the potential to develop as an antifungal drug with different molecular targets.

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Conflict of Interest. None.

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